GJ has many of the same Fs found in red wine, and daily consumption of GJ may reduce the incidence of CAD and APTF by decreasing the platelet contribution to CAD. GJ may be a better source of grape Fs than red wine for those who should not drink alcoholic beverages.

1006-157 Enoxaparin Suppresses Platelet-Dependent Thrombin Generation In Vivo Among Patients With Unstable Angina and Non-Q-Wave Myocardial Infarction

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The potential of low molecular weight heparin (LMWH) to inhibit platelet-rich arterial thrombosis is being actively investigated. We determined the ability of plasma samples from patients with unstable angina and non-Q-wave MI receiving the LMWH Enoxaparin to inhibit tissue factor-initiated thrombin generation in platelet-rich plasma (PRP) and to inactivate platelet prothrombinase. PRP was made by suspending washed donor platelets in the plasma of 7 patients obtained before, 1 h after IV Enoxaparin (30 mg bolus) and 24 h after starting SC therapy (1.0–1.25 mg/kg SC q 12 h). Tissue factor (0.1 ng/ml) and 10 mM $CaCl_2$ were added to initiate extrinsic coagulation. At timed intervals levels of prothrombin fragment 1 + 2 (F1 + 2) were measured by ELISA (thrombin formation). F1 + 2 was decreased by 40% at 1 h and 24% at 24 h (anti-IIa activity). We then assessed platelet prothrombinase inactivation. Prothrombinase was generated using donor platelets, α-thrombin (10 nM), factor Xa (2 nM) and factor Va (2 nM). It was then mixed with patient plasma. Inhibition of prothrombinase assembled on platelets was determined by measuring kinetic changes of F1 + 2 levels. Prothrombinase activity was reduced by 30% at 1 h and 28% at 24 h (anti-Xa activity). We conclude that Enoxaparin in patient plasma is able to: (1) inhibit platelet-dependent thrombin generation, and (2) directly inactivate platelet prothrombinase, indicating that its anti-Xa activity is probably important for its antithrombotic effects.

1006-158 Malondialdehyde Inhibits Platelet-Mediated Vasodilation by Interfering with Platelet-Derived ADP

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Malondialdehyde (MDA) is a peroxidation side-product of thromboxane (TX) synthesis during platelet (plt) activation. TX and MDA are made in increased quantities by plts from patients with atherosclerotic vascular disease and diabetes mellitus. Normal human plts (NL-plts) cause endothelium-dependent vasodilation (EDV) via release of ADP. Since MDA is known to bind to nucleotides, we tested whether it inhibits plt-mediated EDV. *Methods:* Changes in arterial diameter (CIAD) were imaged, digitized and analyzed while NL-plts were thrombin activated and perfused through preconstricted normal rabbit carotid arteries. *Results:* Activated NL-plts perfused with or without concomitant 5 μM MDA (introduced into the perfusate 90 sec after thrombin and plts were mixed) produced 2 ± 1% vs. 12 ± 2% and 1 ± 1% vs. 24 ± 2% CIAD for 5×10^7 and 1×10^8 plts/ml respectively ($n = 6$, $p < 0.01$). When ADP was perfused in incremental doses, with or without 5 μM MDA, it produced: −2 ± 1% vs. 5 ± 1%, −3 ± 1% vs. 11 ± 1% and 36 ± 2% vs. 62 ± 3% CIAD, for 10^{-7} 10^{-6} and 10^{-5} M ADP, respectively ($n = 4$, $p < 0.05$). However, MDA did not impair acetylcholine-mediated EDV, nor vasodilation in response to nitroprusside. Dimethyl thiourea (DMTU) binds MDA. When infused with graded doses of ADP and 5 μM MDA, 25 mM DMTU restored ADP mediated vasodilation. Furthermore, when activated plts were perfused with 5 μM MDA along with 25 mM DMTU, they now produced normal vasodilation. *Conclusions:* MDA appears to inhibit platelet-mediated EDV by interacting with plt-derived ADP. This may promote vasospasm and be of clinical significance in syndromes associated with enhanced platelet TX and MDA synthesis.

1006-159 Genetic Typing of Human Platelet Antigens (HPA-1, 2, 3, 4, 5) Using PCR and an Oligonucleotide Ligation assay in a Semi-Automated System

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Background: HPA-1 (PL^A) has been identified as an inherited risk factor for coronary thrombosis. In this report, we describe the adaptation of a PCR with an oligonucleotide ligation assay (OLA) in a semi-automated technique for genotyping of HPA.

Methods: In the oligonucleotide ligation assay two oligonucleotides are hybridized to target DNA and, if there is perfect complementarity, the DNA ligase covalently joins the oligonucleotides. Only the joined oligonucleotides can be detected in an ELISA assay.

Results: The genotypes determined with the ELISA-based oligonucleotide ligation assay were in 100% concordance with results obtained by conventional allele-specific restriction enzyme site analysis, PCR amplification with sequence-specific primers and phenotyping.

Conclusion: Compared to other methods currently used, the OLA approach has the advantage of high sensitivity, high specificity, and rapid assay performance in addition to the potential of automation and the possibility to use a multiplex PCR with simultaneous amplification of different HPA systems.

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1006-160 Spermine, A Novel Antithrombotic Agent: Prevention of Arterial Thrombosis in a Canine Model

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Background: We have previously reported that of the naturally occurring polyamines (di-amine putrescine, tri-amine spermidine and tetra-amine spermine), the spermine is the most potent inhibitor of *in vitro* platelet aggregation induced by collagen, arachidonic acid or adenosine diphosphate. In the present study we tested the ability of spermine to prevent intravascular thrombus formation in an *in vivo* canine model of electric current induced coronary artery thrombus formation.

Methods: Left circumflex coronary artery was instrumented with a Doppler flow probe and a needle electrode. Partially occlusive thrombus was formed by applying 150 μA of current, which damages the endothelium of the coronary artery and promotes thrombus formation. After development of 50% occlusion of the artery by thrombus, the current was stopped and single bolus dose of spermine (2 mg/Kg, intracoronary) or saline (control) was given. The changes in coronary blood flow were monitored continuously by the Doppler flow probe. Bleeding was assessed by weighing the amount of blood absorbed by a preweighed sponge, placed in an incision that was 5 cm long and 1 cm deep.

Results: In the control dogs ($n = 36$) coronary artery was occluded in 70 ± 11 min after stopping the current. In contrast, the single dose of spermine (2 mg/kg $n = 3$) prevented coronary artery occlusion for > 240 min. However, a lower dose of spermine (1 mg/kg; $n = 3$) was not effective. Platelet counts and deep incisional bleeding were not significantly different between the controls and the treated group.

Conclusion: Data suggests that spermine can selectively inhibit intravascular thrombosis without significantly affecting the other hemostatic parameters. Thus, the effect of spermine potentially identifies a platelet mechanism that may be important in thrombus formation.

1007 Endothelial Function in Health and Disease

Tuesday, March 18, 1997, Noon–2:00 p.m.
Anaheim Convention Center, Hall E
Presentation Hour: Noon–1:00 p.m.

1007-167 Role of Nitric Oxide Synthase and Superoxide Dismutase in Endothelial Dysfunction with Aging

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Aging is a risk factor for cardiovascular disease and associated with endothelial dysfunction. However, its nature is unclear and may involve several factors. We investigated endothelial function with acetylcholine and superoxide dismutase (SOD), expression of endothelial NOS and vascular and plasma SOD activity in aging Wistar rats. Dose-response curves to acetylcholine (ACh, 0.1 nM–10 μM), sodium nitroprusside (SNP, 0.1 nM–10 μM), and SOD (0.075–75 U/mL) were obtained in preconstricted isolated aortic rings from young and old rats (5 and 33 months). Expression of eNOS was measured in native aortic endothelial cells by RT-PCR and SOD activity was analyzed in intact vascular tissue and plasma using a chemiluminescence-